

**INTERNATIONAL STANDARDS FOR  
PHYTOSANITARY MEASURES**

***DIAGNOSTIC PROTOCOLS FOR REGULATED PESTS***

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FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS  
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## INTRODUCTION

### SCOPE

This standard provides specific guidance on the structure and content of diagnostic protocols. It also provides guidance on how these protocols will be initiated, reviewed and published. These protocols describe procedures and methods for the detection and identification of pests that are regulated by contracting parties and relevant for international trade. They are addressed to diagnosticians/diagnostic laboratories performing official tests as part of phytosanitary measures. They provide at least the minimum requirements for reliable diagnosis of the relevant pests.

### REFERENCES

*Determination of pest status in an area*, 1998. ISPM No. 8. FAO, Rome.  
*Export certification system*, 1997. ISPM No. 7. FAO, Rome.  
*Guidelines for a phytosanitary import regulatory system*, 2004. ISPM No. 20. FAO, Rome.  
*Guidelines for surveillance*, 1997. ISPM No.6. FAO, Rome.  
*Guidelines for the notification of non-compliance and emergency action*, 2001. ISPM No. 13 FAO, Rome.  
*International Plant Protection Convention*, 1997. FAO, Rome.  
*Pest reporting*, 2002. ISPM No. 17. FAO, Rome.  
*Requirements for the establishment of pest free areas*, 1996. ISPM No. 4. FAO, Rome.

### DEFINITIONS

At its Seventh session in April 2005, the Interim Commission on Phytosanitary Measures adopted recommendations on the publication of ISPMs in a book format (see ICPM-7 report, paragraph 39 and Appendix II). Each book of ISPMs will contain a glossary chapter, i.e. the *Glossary of phytosanitary terms* (ISPM No. 5) in the relevant language.

The "definitions" section in the present ISPM, once integrated into the book, will not contain any definitions but will refer to the Glossary chapter of the book (ISPM No. 5). However, for the purpose of country consultation, this section contains terms or definitions which are new or revised in the present draft standard. Once this standard has been adopted, the new and revised terms and definitions will be transferred into the Glossary chapter of the book (ISPM No. 5), and will not appear in the standard itself.

#### New terms and definitions

pest detection	The process of finding an organism, either in symptomatic or asymptomatic material.
pest diagnosis	The process of pest detection and pest identification
pest identification	The process of ascertaining the taxonomic identity of an organism.

## **OUTLINE OF REQUIREMENTS**

Diagnostic protocols provide guidance on the diagnosis of specified pests. Information relevant for diagnosis is provided on the specified pest, its taxonomic status and the methods to detect and identify it. Diagnostic protocols contain the minimum requirements for reliable diagnosis of the specified pest and provide flexibility to ensure the methods are appropriate for the range of circumstances of use.

Diagnostic protocols are intended to be used by diagnosticians and are subject to review and amendment to take into account new developments in pest diagnosis. Diagnostic protocols for specific pests are included as annexes to this standard.

## BACKGROUND

The purpose of the International Plant Protection Convention (IPPC, 1997) is to secure common and effective action to prevent the spread and introduction of pests of plants and plant products, and to promote appropriate measures for their control (Article I.1). Proper pest detection and pest identification is crucial for the appropriate application of these measures (see for example: ISPM No. 4: *Requirements for the establishment of pest free areas*; ISPM No. 6: *Guidelines for surveillance*; ISPM No. 7: *Export certification system*; ISPM No. 9: *Guidelines for pest eradication programmes*; and ISPM No 20: *Guidelines for a phytosanitary import regulatory system*). In particular, contracting parties need adequate diagnostic procedures for determination of pest status and pest reporting (ISPM No. 8: *Determination of pest status in an area*; ISPM No. 17: *Pest reporting*), and the diagnosis of pests in imported consignments (ISPM No. 13: *Guidelines for the notification of non-compliance and emergency action*).

NPPOs have produced diagnostic protocols for regulated pests in order to adequately fulfil responsibilities according to Article IV of the IPPC (1997), in particular surveillance, import inspections and export certification. In response to the need for regional harmonization, several Regional Plant Protection Organizations (RPPOs) have developed a significant number of regional diagnostic standards. The Interim Commission on Phytosanitary Measures at its Sixth session subsequently recognized that there was a need for diagnostic protocols also on a global scale within the framework of the IPPC and approved the formation of a Technical Panel on diagnostic protocols for that purpose.

Harmonized diagnostic protocols may support efficient phytosanitary measures in a wide range of circumstances and it is expected that the application of harmonized diagnostic protocols will enhance the mutual recognition of test results and may therefore facilitate trade. These protocols should provide an opportunity for sharing knowledge and should also aid the development of expertise and technical cooperation.

## REQUIREMENTS

### 1. Purpose of diagnostic protocols

Diagnostic protocols may be used in different circumstances that may require test methods with different characteristics. These circumstances may include:

- routine diagnosis of a pest widely established in a country
- general surveillance for pest status
- testing of material for compliance with certification schemes
- surveillance for latent infection by pests
- surveillance as part of an eradication scheme
- detection of a pest in an area where it is not known to occur
- cases where a laboratory detects a pest for the first time
- detection of a pest in a consignment originating in a country where the pest is declared to be absent.

In circumstances such as routine diagnosis, the speed and cost of a test method may be more relevant than sensitivity or specificity, whereas the first detection of a pest in a laboratory or area may require methods with a high level of specificity and reproducibility.

Diagnostic protocols provide the minimum requirements, which may be a single method or a combination of methods, for reliable diagnosis of the relevant pests. Diagnostic protocols also provide additional methods to cover the full range of circumstances for which a diagnostic protocol may be used. The level of sensitivity, specificity and reproducibility of each method is indicated. NPPOs may use these criteria to determine the method or combination of methods that are appropriate for the relevant circumstances. Diagnostic protocols may also be useful in accreditation of laboratories.

### 2. Content of Diagnostic Protocols

Each protocol contains the methods and guidance necessary for the named pest(s) to be detected and positively identified by an expert (i.e. an entomologist, mycologist, virologist, etc.) or competent staff that is specifically trained.<sup>1</sup>

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<sup>1</sup> The following general provisions apply to all diagnostic protocols:

The methods included in diagnostic protocols are selected on the basis of their sensitivity, specificity and reproducibility. In addition, the availability of equipment, the expertise required for these methods and their practicality (for example ease of use, speed and cost) are taken into account when selecting methods for inclusion in the diagnostic protocol. Each diagnostic protocol usually describes more than one method to take into account the capabilities of laboratories and the situations for which the methods are applied. Such situations include diagnosis of different developmental stages of organisms, which require different methodologies, as well as the degree of certainty required by the NPPO.

For some purposes a single method may be sufficient, for others a combination of methods may be necessary. This applies both to the minimum requirements for a diagnosis and where additional requirements are necessary (such as where a high degree of certainty in the diagnosis is required).

Each protocol contains introductory information, information on the taxonomic identity of the pest, methods for detection and identification of the pest, records to be kept, and references to appropriate scientific publications. In many cases a wide range of supplementary information is available, for example on geographical distribution of the pest and host lists, which may support diagnosis, but diagnostic protocols focus on the critical methods and procedures for pest diagnosis.

The aspects of quality assurance that are required by diagnostic protocols (such as inclusion of positive and negative controls) are specifically indicated in the relevant methods in the protocol.

The main elements of the procedure for the development of diagnostic protocols are presented in Appendix 1.

### **3. Structure and Content of a Diagnostic Protocol**

Diagnostic protocols are arranged according to the following sections:

- Introduction
- Taxonomic information
- Detection
- Identification
- Records
- Contact points for further information
- Acknowledgements
- References.

#### **3.1 Introduction**

Brief information is provided on the pest, its appearance, relationship with other organisms, host range (in general), effects on hosts, geographical distribution (in general) and vectors, where appropriate.

#### **3.2 Taxonomic information**

This section provides information on the taxonomy of the pest involved and includes:

- name (correct scientific name and authority (for fungi, teleomorph name))
  - synonyms (including former names)
  - anamorph name of fungi (including synonyms)
  - acronym of viruses.
- taxonomic position (overview of the relevant taxonomic hierarchy).

- Laboratory tests may involve the use of chemicals or apparatus which present a certain hazard. In all cases, local safety procedures should be strictly followed;
- Use of names of chemicals or equipment in these diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable;
- Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated or that proper positive and negative controls are included.

### 3.3 Detection

This section of the diagnostic protocol provides information and guidance on:

- the plants, plant products or other articles capable of harbouring the pest
- the signs and/or symptoms associated with the pest (characteristic features, differences or similarities with signs and/or symptoms from other causes), including illustrations, where appropriate
- the part(s) of the plant, plant products or other articles on/in which it may be found
- the developmental stages of the pest that may be encountered, together with their likely concentration and distribution on/in the plants/plant products or other articles
- the likely occurrence of the pest associated with developmental stages of the host(s) and seasonality
- methods for discovering the pest in the commodity (e.g. visual, hand lens)
- methods for extracting, recovering, and collecting the pest from the plants, plant products or other articles or for demonstrating the presence of the pest in the plants, plant products or other articles. This may include methods for demonstrating the presence of the pest in asymptomatic plant material or other materials (e.g. soil or water), such as ELISA tests or culturing on selective media.

For all the methods included in the section, information is provided on their sensitivity, specificity and reproducibility. Where appropriate, guidance is provided on positive and negative controls and reference material to be included in tests. Guidance is also provided on resolving possible confusion with similar signs and/or symptoms due to other causes.

### 3.4 Identification

This section provides information and guidance on methods that either singly or in combination lead to the identification of the pest. When several methods are mentioned, their advantages/disadvantages are given as well as the extent to which the methods or combinations of methods are equivalent. If several methods are needed to identify the pest a flow diagram may be presented as a figure. Additionally, a flow diagram may be presented if many alternative methods are included.

In cases where morphological methods can be reliably used but appropriate molecular methods have been developed, the latter may be presented as alternative or supplementary methods.

For all the methods included in the section, information is provided on their sensitivity, specificity and reproducibility. Where appropriate, guidance is provided on positive and negative controls and reference material to be included in tests. Guidance is also provided on resolving possible confusion with similar and related species or taxa.

Diagnostic protocols provide guidance on the criteria for the determination of a positive or negative result for each method.

Two main types of methodology are included in diagnostic protocols, methodologies based on morphological characteristics of a pest and those based on biochemical and molecular properties. Morphological characteristics may be investigated directly or may only be examined after culturing or isolation of the pest. This may also be required for biochemical and/or molecular assays. Where culturing or isolation procedures are necessary components of methods, details are provided.

Where appropriate, methods for isolation of pests from asymptomatic plants or plant products (such as tests for latent infection) are given, as well as methods for extraction, recovery and collection of pests from plant or other material. In these cases, methods are also provided for direct identification of pests using biochemical or molecular tests on asymptomatic material.

For morphological identifications, details are provided, as appropriate, on:

- methods to prepare, mount and examine the pest (such as for light microscopy and electron microscopy)
- identification keys (to family, genus, species)

- descriptions of the morphology of the pest or of its colonies, including illustrations of diagnostic characters, and an indication of any difficulties in seeing particular structures
- comparison with similar or related species
- relevant reference specimens or cultures

For biochemical or molecular identifications, each method (e.g. serological methods, BIOLOG, electrophoresis, PCR, TaqMan, RFLP, sequencing) is described separately in sufficient detail (including equipment, reagents and consumables) to be able to perform the test. Where appropriate, reference may be made to methodology described in other diagnostic protocols annexed to this standard.

Cases where the inclusion of appropriate controls, including reference material, is essential (e.g. ELISA) are indicated. When such materials are not available, supplementary tests, preferably based on different identification principles, may increase the certainty of the identification. Ideally, in such cases, a sample, specimen or, where appropriate, an image should be sent to another laboratory with experience in diagnosis of the suspected pest and possessing the required control or reference materials.

Methods for quick, presumptive indications of identity (which will later need to be confirmed) may also be included in diagnostic protocols.

### **3.5 Records**

This section provides information on the records that should be kept:

- scientific name of pest identified
- code or reference number of the sample (for traceability)
- nature of the infested material including scientific name of host where applicable
- origin of the infested material
- description of signs or symptoms (including photographs where relevant)
- methods, including controls, used in the diagnosis and the results obtained with each method
- for morphological methods, measurements, drawings or photographs of the diagnostic features (where relevant), if applicable the developmental stage
- for biochemical and molecular methods, documentation of test results such as photographs of diagnostic gels or ELISA printouts of results, on which the diagnosis was based
- where appropriate, the magnitude of any infestation (how many individual pests found, how much damaged tissue)
- the name of the laboratory and, where appropriate, the name of the person(s) responsible for and/or who performed the diagnosis
- date of diagnostic identification.

Culture(s) of the pest, preserved/mounted specimens or test materials (e.g. photograph of gels, ELISA plate printout results) should be retained in particular in cases of non-compliance (ISPM No. 13): *Guidelines for the notification of non-compliance and emergency action*) and where pests are found for the first time. Additional items may be required under other ISPMs such as ISPM No. 8 (*Determination of pest status in an area*).

### **3.6 Contact points for further information**

Contact details of institutes or individuals with particular expertise on the pest(s) are provided, which may be consulted regarding any questions or for confirmatory diagnosis.

### **3.7 Acknowledgements**

The name and address of the experts who wrote the first draft of the diagnostic protocol are given, together with those of any others who made major contributions.

### **3.8 References**

References to scientific publications and/or published laboratory manuals are given.



#### **4. Publication of Diagnostic Protocols**

Diagnostic protocols are published as annexes to this ISPM and thus are individual publications under the IPPC framework with a specific publication and/or revision date. If appropriate, they may also form part of other ISPMs. The process of their adoption includes stringent review by internationally acknowledged scientists/experts for the relevant discipline.

An index to the annexes is provided as Appendix 2 [Appendix 2 will be added to the standard when protocols have been approved].

## MAIN ELEMENTS OF THE PROCEDURE FOR THE DEVELOPMENT OF DIAGNOSTIC PROTOCOLS

### 1. Production of Diagnostic Protocols

An expert will be commissioned by the Technical Panel on Diagnostic Protocols (TPDP) to lead the development of a diagnostic protocol by adapting diagnostic protocols that have already been approved by RPPOs or other international organizations if they exist, or by developing a new diagnostic protocol. The diagnostic protocol will be produced and reviewed by a small group of experts selected by the TPDP and will then be submitted to the TPDP which, once satisfied with it, will submit it to the standard setting process.

### 2. Review of Existing Diagnostic Protocols

TPDP members will review the diagnostic protocols in their discipline on an annual basis. A request for a revision to a diagnostic protocol may also be submitted to a member of the TPDP or to the IPPC Secretariat (ippc@fao.org), who will in turn forward it to the TPDP.

The TPDP will evaluate the request, identify those diagnostic protocols that require revision and oversee their revision. New methods should be at least as good as existing methods or provide some other advantage. Appropriate evidence should be provided to support any claims.

### 3. Requests for New Diagnostic Protocols

Requests for new diagnostic protocols, in addition to those identified in the work programme of the TPDP, should be sent to the IPPC Secretariat using a form for topics and priorities for standards, by 30 July of each year.